EXPERIMENTAL RESPIRATORY INFECTION WITH POXVIRUSES

I : Clinical Virological and Epidemiological Studies

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Our present understanding of the pathogenesis of the acute viral exanthemata in general, and of smallpox in particular, is based mainly on the classical experiments of Fenner (1948*a*), who used mousepox as a model. He suggested that the pathogenetic processes in many acute exanthemata were basically similar and proposed the following hypothesis. On entering a host a virus multiplies initially at a primary focus ; then an early viraemia is followed by localisation and multiplication in certain internal organs, usually liver, spleen or bone-marrow. All this takes place during the incubation period and symptoms only occur when internal multiplication has reached a sufficiently high titre. At this time the organism is reliberated in large amounts into the bloodstream to produce a secondary viraemia, leading to further foci of infection that may include the endothelium of dermal capillaries or the skin epithelium itself, thus giving rise to the typical rash.

Although the virus studied by Fenner is antigenically closely related to that of smallpox, it differs from the latter in being pathogenic solely to mice, and in entering its host through the skin. Since the portal of entry of variola virus, though still uncertain, is believed to be the respiratory tract, we considered it worthwhile to study respiratory infection with other poxviruses, namely vaccinia, rabbit pox and variola.

Descriptions of the clinical syndromes produced, together with virological and epidemiological data, are reported below. The pathological findings are presented in an accompanying paper.

MATERIALS AND METHODS

Virus Strains

Two strains of rabbit pox virus were used :—The Rockefeller Institute strain (Greene, 1934) which was kindly supplied by Dr. R. E. Shope, and the Utrecht strain (Jansen, 1942) which was obtained from Professor F. Fenner. Both strains were passaged 4 times intratesticularly in rabbits before use and, apart from the slightly greater virulence of the Utrecht strain, gave similar results. The vaccinia virus, which had been propagated for many years on rabbit skin, was received from Professor A. W. Downie. The "Higgins" strain of variola virus came from a fatal case of variola major in the epidemic originating from the S.S. Mooltan (annotation—Lancet, 1949) and was obtained from Dr. F. O. McCallum.

Animals.—Rabbits weighing about 2·5 kg. were used in experiments with vaccinia and rabbit pox, and Indian rhesus monkeys (Macaca Mulatta) for the variola experiments. All animals came from Allington Farm, Porton.

Method of infection.—Animals were exposed to virus in the form of a cloud of dried particles produced in the Henderson apparatus (Henderson, 1952). As about 98 per cent. of such particles were 1μ or less in diameter, the whole of the respiratory tract down to the alveoli

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was exposed to infection. For convenience, dosage of virus has been expressed in terms of pock-forming units (pfu) inhaled, but the actual parameters measured were the time of exposure and the concentration of virus in the cloud. Dosage was then calculated on an assumed inhalation of 800 ml./min. for rabbits and of 1·2 l./min. for monkeys.

Diluent.—This consisted of 0·004 M. McIlvaine buffer (Clarke, 1928) of pH 7·2, containing 1 per cent. normal horse serum, penicillin to a concentration of 250 units/ml. and streptomycin to a concentration of 25 μg./ml.

Virus titrations.—Tissues from animals killed by intravenous "Nembutal" were suspended in diluent, clarified by light centrifugation, and the supernatant fluid titrated on the chorioallantoic membrane of chick embryos (Westwood, Phipps and Boulter, 1957); virus titres are expressed as pfu/g. of original tissue. Blood was usually allowed to clot and then treated as any other solid tissue but, in later experiments specified in the text, the far more sensitive citrated blood technique of Bedson and Duckworth (1963) was used. When necessary, suspensions were stored at −20°; infectivity remained stable for several weeks under these conditions.

RESULTS

The clinical syndromes

Vaccinia.—The response to infection with this virus was very mild. Following an incubation period of 5–6 days, the temperature rose above 104°F., remained elevated for a day or so and then fell to normal. If the skin was shaved a scanty papular rash could be observed around the 6th–7th days; these papules increased in number but rapidly scabbed over. Apart from the pyrexia and an occasional nasal discharge, the unshaven rabbit could not be distinguished from a healthy rabbit.

Rabbit pox.—A far severer reaction was produced by rabbit pox virus. The animal remained apparently healthy during an incubation period of 4–6 days but, as the temperature rose, obvious signs of illness appeared. Listlessness, weakness and rapid loss of weight were followed by profuse, purulent discharges from eyes and nose. A bright erythema of the anal margin preceded the appearance of large pock-like lesions, both at this site and on lips and tongue. Generally the skin was not shaved, but a few experiments with the Utrecht strain indicated that a generalised skin rash occurred about the same time as the lesions on the muco-cutaneous junctions. This rash resembled that of vaccinia in that the lesions increased in number over the next few days, but differed by the marked oedema associated with the individual lesions. The extent of the rash varied from a few scattered lesions to actual confluency; in some cases death occurred before a rash could develop. A lesion began as a red papule; the later development of a yellow centre converted it to a pseudo-pustule whose contents were caseous rather than true pus; finally it disappeared without scarring. The surface epithelium of lesions sometimes became scaly but true scab formation was absent. Death usually occurred between the 7th and 12th days after infection and was signalled by a fall in body temperature to subnormal levels, occasionally so low as to be unrecordable with the clinical thermometer.

Variola.—Respiratory infection of monkeys with variola virus produced a disease of severity intermediate between that of vaccinia and rabbit pox in rabbits. Overt signs of illness coincided with the onset of pyrexia, after an incubation period of about 5 days. The monkey huddled quietly in a corner, food was ignored and, on handling, a marked weakness and loss of muscle tone was evident. A rash occurred usually on the 7th or 8th days after infection, but varied from the 6th–11th days. It followed the centrifugal distribution typical of human smallpox. Signs of recovery, as shown by increased liveliness and a return of

appetite, tended to coincide with a fall in body temperature to normal levels. There were only 2 deaths out of 109 monkeys infected; they occurred on the 10th and 11th days after infection respectively and both showed the same profound fall in body temperature that was noted in rabbit pox.

Virological studies

Infectivity by the respiratory route.—The infectivity of vaccinia and rabbit pox viruses was investigated by exposing rabbits to aerosols of decreasing concentrations of the relevant virus. Successful infection with rabbit pox virus was judged by the subsequent development of pyrexia and other clinical signs of disease. For vaccinia virus, actual proof of viral multiplication was sought by titrating the virus present in the lungs 6 days later; it was found that virus was either totally absent or present in amounts at least 500 times more than the total inhaled dose.

TABLE I.—*The infectivity of Vaccinia and Rabbit Pox Viruses for Rabbits by the Respiratory Route*

Dose (pfu) (inhaled)	Vaccinia Number of Rabbits			Rabbit Pox Number of Rabbits		
	Exposed	Infected	Died	Exposed	Infected	Died
4	3	3	0	*	*	*
8	3	2	0	*	*	*
15	6	2	0	7	7	6
30	6	6	0	*	*	*
50	3	3	0	8	8	7
100	3	3	0	*	*	*
250	2	2	0	9	9	8

* Signifies no animals exposed at that dose level.

The great infectivity of both viruses is evident in Table I; in each case the lowest dose used was capable of initiating infection. Moreover, the doses given in the table refer to the total virus inhaled, of which only a proportion is retained in the lungs. Accurate data on the distribution of inhaled particles, of the size range involved in these experiments, are not available for the rabbit. Similar data for monkeys and guinea-pigs (Harper and Morton, 1953), and for mice (Harper and Hood, 1962), suggest that only 25 per cent. of inhaled particles are actually retained in the lungs; the remainder is either exhaled (25 per cent.) or passes to the alimentary tract (50 per cent.). Since we were unable to infect rabbits by feeding even large doses of virus by stomach tube, it appears that infection is initiated solely by the 25 per cent. retained in the lungs and that a single pfu, of vaccinia virus is sufficient for this purpose. Although the dosage of rabbit pox was not taken quite so low as with vaccinia, the results suggest that there is little, if any, difference between the viruses in infectivity. Despite the great infectivity of vaccinia virus, we were unable to produce a fatal disease, or even a severe infection, with doses as high as 13,000 pfu.

Growth of virus in the lungs.—In these experiments the initial site of viral multiplication was the respiratory tract and it can be seen from the Fig. that there was no difference between vaccinia and rabbit pox in their rate of multiplication at this site, although the latter virus reached a maximum titre which was tenfold

higher than that of vaccinia. In both cases titres had begun to fall by the 10th day but rabbit pox remained detectable for longer period than vaccinia. Although sampling was less frequent in the variola experiments the results presented in Table IV show that this virus was also capable of multiplying to high titre in the lungs.

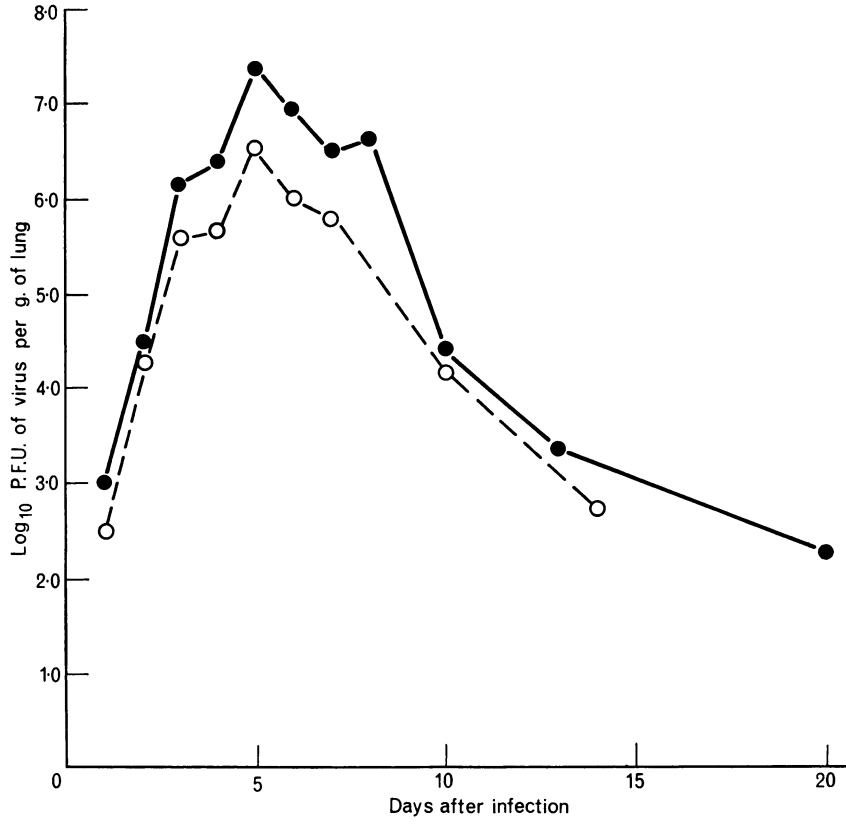

FIG.—Multiplication of vaccinia and rabbit pox viruses in rabbit lungs ●—● rabbit pox. ○——○ vaccinia

Growth of virus in other tissues.—In order to follow the spread of virus from the primary focus in the lungs to other parts of the body, animals were killed at intervals and tissues removed for virus titration. The results are presented in Table II (vaccinia), Table III (rabbit pox) and Table IV (variola).

A stepwise progression from primary focus to draining lymph node and then to generalised dissemination occurred with rabbit pox. The initial involvement of lymphatic pathways was not evident with the other viruses but this may be explained by the failure to sample before the 3rd day in the variola experiments and, in the vaccinia experiments, by the difficulty in differentiating lymph node from surrounding adipose tissue before the 4th day, when a reactive hyperplasia made this task easy.

Despite the similar multiplication rates of all three viruses in the lungs, there

TABLE II.—*The Distribution of Vaccinia Virus in Rabbit Tissues at Intervals After Respiratory Infection*

Tissue	Expt. No.	Virus titre at daily intervals: 0	1	2	3	4	5	6	7	10	14	21	28
Lung	1	*	1·9	4·1	5·1	5·1	*	5·9	4·8	*	2·8	*	*
		*	2·7	4·3	5·3	5·6	*	6·4	5·3	*	4·0	—	—
	2	—	2·7	4·4	6·2	6·6	6·7	5·3	6·3	3·9	—	—	—
		1·4	2·7	4·5	5·8	5·4	6·5	6·5	6·8	4·5	4·2	—	—
Hilar lymph node	1	*	*	*	*	*	*	2·0	—	*	—	*	*
		*	*	*	*	*	*	—	—	*	—	—	—
	2	—	—	—	—	3·1	2·8	2·6	1·1	—	—	—	—
		—	—	—	—	2·4	1·7	—	3·0	—	—	—	—
Blood	1	*	—	—	—	—	*	—	—	*	—	*	*
		*	—	1·0	—	—	*	—	—	*	—	—	—
	2	—	—	—	—	—	—	—	2·3	—	—	—	—
		—	—	—	—	—	—	—	—	—	—	—	—
Spleen	1	*	—	—	—	—	*	—	—	*	—	*	*
		*	—	1·4	—	—	*	—	—	*	—	—	—
	2	—	—	—	—	—	—	1·4	2·0	—	—	—	—
		—	—	—	—	—	—	—	—	—	—	—	—
Liver	1	*	—	2·4	—	—	*	—	—	*	—	—	—
		*	—	2·9	—	—	*	—	—	*	—	—	—
	2	—	—	—	—	—	—	—	2·9	—	—	—	—
		—	—	—	—	—	2·63	—	1·4	—	—	—	—
Kidney	1	*	—	1·3	—	—	*	—	—	*	—	*	*
		*	—	—	—	1·4	*	—	—	*	—	—	—
Adrenal	1	*	—	—	—	—	*	—	—	*	—	*	*
		*	—	—	—	—	*	—	—	*	—	—	—
	2	—	—	—	—	—	—	—	1·0	—	—	—	—
		—	—	—	—	—	—	—	—	—	—	—	—
Gonad	1	*	—	1·5	—	—	*	1·2	—	*	—	*	*
		*	—	1·0	—	—	*	—	—	*	—	—	—
	2	—	—	—	—	—	—	3·7	3·4	—	—	—	—
		—	—	—	—	—	—	—	—	—	—	—	—
Thymus	1	*	*	*	—	—	*	1·0	—	*	—	*	*
		*	*	*	—	—	*	0·4	—	*	—	—	—
Brain	1	*	—	1·0	—	—	*	—	—	*	—	*	*
		*	—	1·8	—	—	*	—	—	*	—	*	*
Skin	2	—	—	—	—	—	—	—	2·1	—	—	—	—
		—	—	—	—	—	—	—	1·6	2·2	—	—	—

Figures represent titre of virus expressed as $\log_{10}$/g. of wet tissue (or ml. in case of blood). In any one column the virological findings in the tissues of the same rabbit are always placed in the same relative position.

— Indicates no virus detected. * Indicates tissue not examined.

were marked differences in their ability to multiply in other tissues. Thus, although vaccinia virus occurred at one time or another in every tissue examined, it did so only sporadically and in low titre ; there was no evidence of the regular presence or multiplication of the virus anywhere other than the primary focus. In contrast, rabbit pox virus was readily recovered from a variety of tissues from the 4th day onwards. Some interesting differences in viral replication in various organs are evident in Table III. In liver and spleen a brief period of multiplication was followed by an abrupt disappearance of virus, whereas continued multiplication to very high titres occurred in adrenals and gonads. Apart from the early disappearance of virus from spleen and liver, a general fall in titre began after the 8th day ; after the 27th day virus could not be recovered from any tissue. The results of the variola experiments, with 2 exceptions, give an overall picture resembling that of vaccinia in rabbits, namely high titres of virus at the primary

TABLE III.—*The Distribution of Rabbit-Pox Virus in Rabbit Tissues at Intervals After Respiratory Infection*

Tissue	Virus titre at daily intervals											
	1	2	3	4	5	6	7	8	10	13	20	27
Lung	2·6	4·5	6·2	5·9	7·4	8·1	6·2	6·8	6·0	3·9	4·5	—
	3·4	4·6	6·2	6·9	7·5	5·8	6·7	6·4	2·8	2·9	—	—
Hilar lymph node	—	2·1	3·6	4·2	5·4	3·5	3·2	2·2	—	—	—	—
	—	—	3·0	4·3	4·4	2·8	4·5	—	—	—	—	—
Spleen	—	—	—	3·3	5·2	6·1	—	—	—	—	2·1	—
	—	—	—	3·3	4·3	—	—	—	—	—	—	—
Liver	—	—	—	2.7	4·6	4·7	—	—	—	—	—	—
	—	—	—	2·6	3·1	—	—	—	—	—	—	—
Kidney	—	—	—	—	3·3	2·8	—	1·9	2·6	2·6	—	—
	—	—	—	—	2·4	—	—	—	2·4	—	—	—
Adrenal	—	—	—	3·8	4·2	6·8	7·3	7·1	5·5	2·7	3·0	—
	—	—	—	2·5	3·6	5·2	5·2	4·0	—	—	3·4	—
Gonad	—	—	—	—	5·7	6·1	6·4	6·8	6·8	4·9	5·3	—
	—	—	—	3·6	3·1	5·4	6·1	7·3	3·4	5·4	3·2	—
Brain	—	—	—	—	—	2·4	—	2·1	—	—	—	—
	—	—	—	1·6	1·2	—	—	—	—	—	—	—
Skin	—	—	—	—	3·2	—	3·7	5·7	3·7	2·0	4·4	—
	—	—	—	—	—	—	2·0	6·2	—	2·8	—	—

Figures represent titre of virus expressed as $\log_{10}$ pfu/g of wet tissue. In any one column the virological findings in the tissues of the same rabbit are always placed in the same relative position.

— Indicates no virus detected.

focus but only sporadic occurrence in low titre in other organs. The exceptions are the samples removed on days 10 and 11 ; these came from the only two fatal cases in the series and are therefore probably not representative of the general distribution of virus at this late stage of disease. The samples on day 10 came from a very sick monkey which collapsed and died a few moments after it was returned to the cage. The presence of large amounts of virus in lungs and skin, but complete absence from other tissues examined, is puzzling and no explanation can be offered. The samples removed on day 11, which contained the greatest amounts of virus ever found, came from a monkey found dead on that morning ; this particular animal had a concurrent Flexner dysentery infection.

Viraemia.—The occasions on which virus was recovered from ground-up blood clots are listed in Table V. Variola was never found in monkey blood and the other two viruses were only detected irregularly between the 5th and 8th days after infection.

More recent experiments, using the citrated blood technique of Bedson and Duckworth (1963), have shown that clotted samples grossly underestimate the amount of virus circulating in the bloodstream. The " in vitro " addition of virus to blood indicates that a recovery of about 50 per cent. of added virus is obtained from citrated blood, whereas only 0·1 per cent is recovered from clotted blood (Maber—unpublished observations) ; presumably there is gross adsorption of virus to fibrin during the clotting process. The results of 3 experiments with rabbit pox are presented in order of time of death in Table VI. This order of presentation reveals the presence of 2 distinct patterns of viraemia. Animals dying early had viraemias which increased exponentially to reach very high levels at death, whereas those dying later had much lower and more constant levels. It is of interest to note that, while rabbits surviving infection tended to have the lowest degree of viraemia, the complete absence of a detectable viraemia did not

TABLE IV.—*Distribution of Variola Virus in Monkey Tissues at Intervals After Respiratory Infection*

Tissue	Virus titre at daily intervals											
	0	3	7	8	9	10	11	12	15	20	25	35
Lung	—	7·0	4·9	4·6	—	7·2	9·4	—	—	—	—	—
	—	6·2	4·0	5·2	—	*	*	—	—	*	—	—
	*	6·6	4·5	5·6	7·7	*	*	*	*	*	—	*
	*	7·2	3·4	*	4·2	*	*	*	*	*	*	*
Hilar lymph node	*	*	—	—	—	—	*	—	—	*	—	*
	*	*	—	—	—	*	*	—	*	*	—	*
	*	2·5	—	—	4·8	*	*	*	*	*	*	*
Blood	*	—	—	*	—	*	*	*	*	*	*	*
	*	—	—	*	*	—	—	—	*	—	*	—
	*	*	—	*	*	*	*	—	*	*	*	—
	*	*	*	*	*	*	*	*	*	*	—	*
	*	*	*	*	*	*	*	*	*	*	*	*
Spleen	*	—	2·5	—	—	—	7·1	—	—	*	—	*
	*	2·2	1·6	—	—	*	*	—	*	*	—	*
	*	—	—	—	—	*	*	*	*	*	*	*
	*	—	—	—	—	*	*	*	*	*	*	*
Liver	*	—	—	—	—	—	7·6	—	—	*	—	*
	*	—	—	—	—	*	*	—	*	*	—	*
	*	—	—	—	—	*	*	*	*	*	*	*
	*	—	—	*	—	*	*	*	*	*	*	*
Adrenal	*	—	—	—	—	—	6·0	—	—	*	—	*
	*	—	—	—	—	*	*	—	*	*	—	*
	*	—	—	—	—	*	*	*	*	*	*	*
	*	—	—	*	—	*	*	*	*	*	*	*
Skin	*	*	4·0	*	*	5·4	*	*	*	*	*	*
	*	*	*	*	*	*	*	*	*	*	*	*
	*	*	4·4	*	*	*	*	*	*	*	*	*
	*	*	*	*	*	*	*	*	*	*	*	*
Gonad	*	—	—	—	—	*	6·4	—	—	*	—	*
	*	—	—	—	—	*	*	—	*	*	—	*
	*	—	—	—	4·7	*	*	*	*	*	*	*
	*	—	—	*	—	*	*	*	*	*	*	*

Figures represent titre of virus expressed as $\log_{10}$ pfu/g of wet tissue (or ml. in the case of blood). In any one column the virological findings in the tissues of the same monkey are always placed in the same relative position.

— Indicates no virus detected. * Indicates tissue not examined.

preclude death (Rabbit 12—Table VI). In a further experiment, in which the virus dose was increased a 100-fold, the same dual pattern was still evident although survival time was much shortened (Table VII). In all these experiments the onset of viraemia was closely associated with the onset of pyrexia ; in a total of 17 rabbits, one had viraemia 24 hr. before pyrexia, 9 were synchronous with pyrexia, 5 began 24 hr. after pyrexia and 2 were delayed until 48 hr. after pyrexia.

TABLE V.—*Recovery of Virus from Blood Clots*

Virus	Days after infection											
	1	2	3	4	5	6	7	8	9	10	11	12
Vaccinia	0/3	0/5	0/4	0/2	1/5	1/5	1/5	1/3	0/3	0/5	*	0/3
Rabbit pox	0/3	0/3	0/3	*	2/3	1/3	0/3	1/3	0/3	0/3	*	0/3
Variola	0/12	0/11	0/12	*	0/12	*	*	*	0/1	*	*	*

Figures represent No. Positive, No. examined.
* = No specimen examined.

TABLE VI.—*Daily Blood Levels of Rabbit Pox Virus in Fatal and Non-fatal Infections*

Rabbit No.	Days after infection									
	3	4	5	6	7	8	9	10	11	12
1	—	2·8	3·6	4·4	4·5	D	*	*	*	*
2	—	2·4	3·3	4·2	4·3	D	*	*	*	*
3	—	3·1	3·9	5·0	5·7	D	*	*	*	*
4	—	2·7	3·9	4·4	5·0	D	*	*	*	*
5	—	2·3	2·5	2·6	2·6	2·5	D	*	*	*
6	—	2·5	3·1	3·3	3·5	2·7	D	*	*	*
7	—	1·4	2·9	3·1	3·4	3·5	D	*	*	*
8	—	2·3	2·6	2·8	—	1·7	*	D	*	*
9	—	—	—	1·5	3·3	3·2	*	D	*	*
10	—	—	—	3·4	3·2	2·7	*	D	*	*
11	—	2·4	2·9	2·6	2·5	2·6	*	D	*	*
12	—	—	—	—	—	—	*	—	D	*
13	—	2·1	2·7	2·7	3·0	3·0	*	2·8	1·9	—†
14	—	—	2·5	2·7	2·7	2·6	*	2·2	1·3	—S
15	—	1·4	1·4	1·4	—	—	*	—	—	—S
16	—	2·1	2·1	1·7	2·5	2·2	*	1·9	—	—S
17	—	2·2	—	1·7	1·9	1·8	*	—	—	—S
18	—	—	2·0	2·9	3·2	1·9	*	—	—	—S

Figures represent titres expressed as Log_{10} pfu/ml. blood.
— = Negative. (20 pfu/ml. blood).
* = Not examined.
D = Died.
† = Died on Day 13.
S = Survived.

TABLE VII.—*Daily Blood Levels of Rabbit Pox Virus After Massive Infection*

Rabbit No.	Days after infection						
	3	4	5	6	7	8	9
R 35	3·3	5·7	D	..	..	..	..
Q 189	2·6	4·4	D	..	..	..	..
Q 154	2·8	3·3	D	..	..	..	..
Q 128	2·6	3·6	6·4 D	..	..	..	..
Q 142	2·7	4·4	D	..	..	..	..
Q 160	2·4	2·8	3·3	D	..	..	..
Q 146	2·2	3·0	4·4	D	..	..	..
R 20	1·5	2·6	2·6	2·9	2·3	2·2	D

Figures represent titres expressed as Log_{10} pfu/ml. blood.
D = Dead.
NB—Rabbit Q128 died shortly after bleeding on Day 5.

Epidemiological studies

The excretion of virus.—The profuse ocular and nasal discharges that occur in rabbit pox suggest an obvious mechanism for the spread of infection to fresh animals. The degree to which virus is excreted by these routes at different stages of the disease was therefore investigated. Sterile cotton wool pledgets, mounted on sticks, were immersed in 1·0 ml. of diluent. After being freed from excess fluid by squeezing against the side of the tube, a swab was rubbed gently over the eyelids or anterior nares and replaced in the fluid, where it was first agitated to disperse the collected material and then squeezed as dry as possible ; the fluid was later titrated for virus in the usual way. The combined results of 5 experiments are summarised in Table VIII.

TABLE VIII.—*The Excretion of Rabbit Pox Virus in Ocular and Nasal Discharges*

		Days after infection									
		3	4	5	6	7	8	9	10	11	12
Ocular discharges	No. samples[1]	0/23	1/23	9/23	17/23	17/21	6/10	ND	5/9	4/7	2/5
	Mean titre[2]	—	1·6	1·3	2·3	2·7	3·3	—	3·3	2·6	2·3
	Max titre	—	—	3·0	4·0	4·5	5·4	—	4·6	4·8	4·3
	Min. titre	—	—	0·4	0·8	0·3	0·6	—	2·0	0·4	0·3
Nasal discharges	No. samples	1/10	2/10	9/10	10/10	10/10	ND	ND	5/5	3/3	2/2
	Mean titre	1·0	1·2	1·9	3·6	4·5	—	—	4·8	3·8	2·4
	Max. titre	—	1·5	3·9	4·8	5·6	—	—	6·1	5·8	2·7
	Min. titre	—	0·9	0·4	2·2	3·8	—	—	3·9	2·5	2·1

[1] Figures represent No. positive/No. examined.
[2] Titres expressed as Log_{10} pfu/ml. (see text.)

Since neither the total volume of exudate, nor the amount picked up on the swab was known, Table VIII gives only a rough estimate of the amount of virus excreted by these routes. The discharge began as a slight moistening of the surrounding fur about 1 or 2 days after the onset of pyrexia, but soon became copious and purulent. The only differences between the 2 routes were that the proportion of excretors and the concentration of virus excreted were greater for nasal discharges. The degree of environmental contamination is obviously a function of the numbers of rabbits excreting virus, the volume of the discharge and the concentration of virus in the discharges. It appears to be maximal about the 6th and 7th days since, although the volume and virus titre of individual discharges remains high, the number of excreting rabbits falls rapidly after day 7 because of deaths.

Air sampling.—Several attempts to isolate rabbit pox virus from the air of rooms containing infected animals were made. Most of the sampling was done with an electrostatic precipitator (Morris, Darlow, Peel and Wright, 1961), but duplicate samples were sometimes collected in a raised glass impinger (May and Harper, 1957). The results of 7 experiments are summarised in Table IX. Virus recovery was confined to the 6th and 7th days after infection, and the concentration of virus on these occasions never exceeded 4 pfu./90 l. of air. Although Morris (unpublished observations) has shown that impingers and precipitators are of similar efficiency in recovering vaccinia virus from air, our only successes were obtained with the latter instrument ; this presumably reflects the greater volume of air sampled by the precipitator.

TABLE IX.—*Recovery of Rabbit Pox Virus from Air of Rooms Containing Infected Rabbits*

	Days after infection									
	3	4	5	6	7	8	9	10	11	12
Number of samples tested	1	1	2	3	7	3	2	5	4	2
Number of samples positive	0	0	0	2	2	0	0	0	0	0

Cross-infection studies.—To obtain information on the spread of disease from rabbit to rabbit, normal animals were placed in the same room as animals infected with rabbit pox virus. The rabbits were never in actual contact with each other,

but were caged separately ; the cage to cage distance varied from a few inches to as much as 12 ft. When a rabbit failed to become infected during the course of an experiment it was left in the room during subsequent experiments until infection occurred. These " indicator " rabbits were present from the first day of infection of the ordinary " experimental " rabbits. All of 7 such "indicator " rabbits eventually contracted rabbit pox and it was found that the interval between onset of disease and the last introduction of infected " experimental " rabbits ranged from 11–16 days.

DISCUSSION

The disease patterns arising from respiratory infection of rabbits with vaccinia and rabbit pox, and of monkeys with variola, were basically similar to that found by Fenner (1948*b*) in cutaneous infection of mice with ectromelia. Although Hahon (1961) has suggested that Fenner's hypothesis on the pathogenesis of acute exanthemata is only relevant to poxvirus infections in which the virus enters a host through the skin, our own results, like those of Bedson and Duckworth (1963), indicate that it has a more general validity. The differences that do exist between various virus-host systems are differences of detail and seem unrelated to the route of infection.

All three viruses were infective by the respiratory route, and the data on vaccinia and rabbit pox suggest that little more than one infective particle is needed for infection. Fluorescent antibody studies with vaccinia (Lancaster, Boulter, Westwood and Randles 1966) show that the cells initially involved are those of the bronchiolar epithelium and alveoli. Once infection was initiated the viruses multiplied in the lungs at similar rates to reach high titres.

The involvement of lymphatic pathways in the spread of virus from the primary focus was most evident in the rabbit pox experiments. Although dissemination from the lymph nodes was presumably through the bloodstream we were unable to detect a viraemia before the 4th day after infection, by which time virus was multiplying in many tissues. In this we agree with previous reports for rabbit pox (Bedson and Duckworth, 1963), mousepox (Fenner, 1948*b*) and variola (Hahon and Wilson, 1960) ; we differ only in the finding of two distinct patterns of viraemia that were related to the time of death. In an earlier paper (Boulter, Maber and Bowen, 1961) we reported that early deaths in rabbit pox differed from late deaths by the presence of a blood coagulation defect ; the discovery that they also differ in having a progressively increasing viraemia that attains massive proportions leads us to suggest that such early deaths are analogous to the severe purpuric or haemorrhagic forms of smallpox. The viraemia of rabbit pox resembles that reported for smallpox (Downie, McCarthy, Macdonald, McCallum and Macrae, 1953) in its occurrence at the onset of overt disease, in the direct relationship between titre and severity of disease, and in its absence in some fatal cases.

Despite the similarities between the viruses in their infectivity and their rates of replication in the lungs there were marked differences in their ability to multiply in organs other than lung. The degree of extra-pulmonary multiplication was related to pathogenicity. Thus vaccinia, producing only a brief pyrexia, was never found in high titre outside the lung, although present sporadically in every tissue examined. In contrast, the virulent rabbit pox virus became widespread throughout the body, often reaching titres in excess of 10^6 pfu./g. Apart from

the two fatal cases, variola in monkeys resembled vaccinia in rabbits in the failure to attain high titres outside the primary focus. The similar results of Hahon and Wilson (1960), and the mild nature of the disease, suggest that monkey variola is a less valid model of human smallpox than the more distant but more lethal, diseases of rabbit pox and mousepox.

The early disappearance of rabbit pox virus from liver and spleen is of interest, particularly as these organs are the major sites of internal viral multiplication in mousepox. In the case of spleen, this could result from local antibody production but the longer persistence of virus in lymph node argues against this explanation. Another point of note is the involvement of adrenals and gonads in rabbit pox ; although we did not match our animals for sex it seemed immaterial whether the gonad was ovary or testis. One factor common to all three organs is their involvement in steroidal hormone synthesis and the effect of such hormones on susceptibility to infections is well documented. Whether this finding is peculiar to rabbit pox or has relevance to other pox diseases is uncertain, and there is clearly a need for more information on the distribution of virus in the tissues of fatal cases of human smallpox.

Bedson and Duckworth (1963), by caging normal rabbits with infected rabbits for 24 hr. periods at different stages of the disease, found that rabbit-to-rabbit spread occurred only when nasal and conjunctival discharges were present. Our own results, obtained by different methods, confirm the importance of these discharges in the spread of infection and show that actual contact is not essential ; transmission can occur across the width of a room. The studies on virus excretion suggest that environmental contamination was maximal 6–7 days after infections and it was only on those two days that virus was recovered by air-sampling techniques. Assuming an incubation period of 4–6 days, normal rabbits would thus be expected to become ill between 10 and 13 days after the introduction of freshly infected rabbits to the same room ; in practice, the period was found to range from 11–16 days. The small amounts of virus recovered from the air samples suggest that aerial contamination may be intermittent and of low degree, but the actual degree of contamination is difficult to assess since a plenum plant exchanged air at the rate of one-room-volume every six minutes. Meiklejohn, Kempe, Downie, Berge, St. Vincent and Rao (1961) had similar difficulty in recovering variola virus from the air of a smallpox ward, even when sampled within one foot of a patient's mouth or close to infected sheets which were being shaken. However, the sampler used (tightly packed cotton) was not the most efficient and it would be of value to repeat their experiments, using a method as sensitive as the electrostatic precipitator. That the information to be gained is of more than academic interest is indicated by the recent occurrence of 4 cases of smallpox in England and Wales whose only known exposure was that they all came within 10,000 ft. of a smallpox hospital (Bradley, 1963). Two of these cases gave rise to minor epidemics involving 44 further cases and 19 deaths (Culley, 1963). The long-standing controversy over aerial transmission of variola is well reviewed by Dixon (1962) ; the consensus of opinion has been that clandestine contact is always the mechanism involved but recent data (Westwood, 1963) suggests that airborne spread is a real, if unusual, hazard. In this respect it is emphasised that even the most efficient sampler cannot compare with a human being, who samples air continuously at the rate of 10 l. per min., and is probably sensitive to a single particle.

SUMMARY

Vaccinia, variola and rabbit pox viruses all proved highly infectious to animals by the respiratory route and multiplied at similar rates in the lungs. The former two viruses failed to replicate to any great degree in any site other than the primary focus, but rabbit pox produced a fatal generalised infection characterised by high titres of virus in adrenals and gonads. The viruses spread by lymphatic pathways to the regional nodes and thence by the bloodstream to the rest of the body. Two distinct patterns of viraemia, associated with early and late deaths respectively, were noted in rabbit pox.

Rabbit pox also differed from the other two viruses in its ability to spread by aerial routes to other animals. Virus was actually recovered by air-sampling techniques and probably arose from infected oculo-nasal discharges. The implications of these findings in relationship to human smallpox are discussed.

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ADDENDUM

In a further report on the recovery of smallpox virus by air-sampling methods, in which fluid impingers and settling plates were used (Downie, Meiklejohn, St. Vincent, Rao, Sundara Babu and Kempe, 1965), it was concluded that contamination of the air in the vicinity of smallpox patients was primarily due to comparatively large particles.

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